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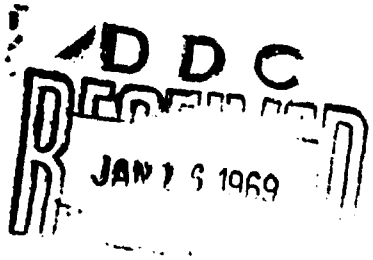
SMUFD, D/A ltr, 17 Feb 1972

TRANSLATION NO. 2359

DATE: Nov 1968



DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

Translation No. [redacted]

Author: Dr. Bernd Liess, Groner Landstrasse 2, Gottingen, Germany

Title: Observations and investigations on contagious pustular dermatitis (Ecthyma contagiosum) of sheep as a human disease (Beobachtungen und Untersuchungen uber den Lippengrind (Ecthyma contagiosum) der Schafe als Zooanthroponose).

Journal: Journal for Bacteriology, Parasitology, Infectious Diseases, and Hygiene (Zentralblatt fur Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene) Abt. 1 Orig. 185: 289-304. (1962)

November 1968

In the course of the classroom and field curriculum of the Hannover Veterinary School, a group of ten veterinary medical students treated the lambs of the well-screened sheep herd against tape worm infection by oral administration of Kamala. Capsule forms of the medication were manually pushed over the back of the tongue without the help of instruments, and in this way, the animal was forced to absorb it. During these manipulations, the opportunity was present for the students to injure themselves on the teeth of the sheep. In four cases that were observed, many days later at the site of skin injury on the hand, exanthemata appeared which caused confusion at the Institute for Microbiology and Animal Epidemics of the Hannover Veterinary School. From the view point of human dermatology, a diagnosis of "sheep pox" was made. The pock-like nature of the disease in conjunction with the amnestically raised epidemiological relation to sheep may be responsible for this. The infections, however, were believed to be caused by the virus of pustular dermatitis of sheep and goats (Ecthyma contagiosum). The clinical appearance as well as the apparent infection via sheep are described in the case histories of two of the patients as follows:

Patient H.

14 or 15 July 1959

Bitten on the finger by a sheep in the Ecthyma-suspected herd; poor wound healing followed.

27 or 28 July 1959

Wound site strongly inflammed; initial blister formation - increasing wound swelling and blister enlargement.

1 or 2 August 1959

At the site of the blister, there appeared a pustule surrounded by inflammation; Pustule enlargement.

5 August 1968

Admission to the Institute. On 5 August 1959, 20 or 21 days after the presumed infection, the exanthema appeared as an approximately pea-shaped, compact pustule with a yellowish-white covering and surrounded by bluish-red inflammation. By means of an incision, the covering of the pustule was shown to be about 0.5 mm thick. The pustule contents consisted of a clear serous fluid.

Patient L.

14 or 15 July 1959

Bitten on fingers by sheep in the Ecthyma-suspected herd.

21 or 22 July 1959

Conspicuous skin lesions in the vicinity of the wound made by the bite.

24 July 1959

Admitted to the Institute. On 24 July 1959, the exanthemata consisted of individual seed to pea-sized pustules on the forefinger of the right hand and in the interdigital spaces. By 13 August 1959, and consequently within 30 days after the suspected infection via sheep, healing had occurred following involution of the pustule.

In two other students, who had also participated in the sheep treatment, pustular, pock-like skin lesions suggesting milker's nodes were seen. These cases were similar to those described above.

On 29 July 1959, examinations were carried out on the sheep herd in order to detect sequelae of importance. At this time, it was about 13 to 14 days after the time of the probable infection of the students. The shepherd, who was already very familar with pustular dermatitis, pointed out several yearling lambs with infections later diagnosed as contagious pustular dermatitis. On the lips of

these lambs were found crusty depositions typical of Ecthyma contagiosum. These could be removed without inducing strong bleeding leaving behind a small area of damaged epithelial tissue. The crusts were stored for the later virological investigations.

VIROLOGICAL INVESTIGATIONS

Material and Methods

(A) Virus

The starting material of human origin for virological-cultural investigations was obtained from two patients:

(1) From Patient H. (= Virus material H) 20- 21 days post-infection.

(2) From Patient M. (Virus material M) 9 - 10 days post infectior

Virus material H consisted of about 0.2 cm^3 of the blister fluid which had been removed with a capillary pipette. After removal, the fluid was frozen at -30°C until the beginning of virus culture studies 24 hours later. For inoculation, the fluid was diluted in about 1 cm^3 of phosphate buffer solution (Dulbecco and Vogt, 1954).

Virus material M. was obtained by excision of a pustule and was stored for ca. 3½ months at - 70°C until the beginning of virus culture studies. Before inoculation, the tissue material was mixed with ca. 2 cm^3 of phosphate buffer solution, ground with sterile sand, and then centrifuged for 10 minutes at 3,000 rpm. The supernatant was used as the material for inoculation.

The initial material from sheep consisted of skin crusts from naturally ecthyma-infected lambs belonging to the herd which was the source of infection for Patients H. and M. as well as for the other persons infected. Promptly after sampling and without further cultivation, the epidermal crust material was rubbed onto a scarified area of the upper lip of a sheep. On the sixth day after the introduction of the experimental infection upon appearance of the pimples, blisters,

skin pustules characteristic of ecthyma infections, the skin area which had been treated was removed with scissors and scapel, placed in cold physiological saline along with the bloody wound secretions, and ground in a mortar and pestle. The best positive results were obtained when sheep were infected on the lip after serification using crude extracts prepared as described above (Table 1). The ecthyma skin crust obtained from Study Sheep VII was later employed as the starting material for virus cultivation studies in calf testes tissue cultures and was treated treated as follows:

After grinding in a mortar and pestle, a 3 % solution of the material was prepared in phosphate buffer. The material was centrifuged for 10 minutes at 3,000 rpm and 200 units of Mycostatin were added to each cm^3 of supernatant (= virus material Sheep VII).

Ecthyma skin crusts from Study Sheep III were also employed for viral culture studies in embryonated hen eggs after being ground, suspended, and centrifuged. The supernatant was mixed with an equal volume of antibiotic mixture (5,000 units penicillin and 100 mg streptomycin per cm^3), held for 30 minutes at room temperature and then used for inoculation. The inoculum was designated Virus Material-Sheep III.

(B) Viral Cultural Studies in the Allantochorion of Embryonated Hen Eggs.

Viral cultivation studies were carried out using the chorioallantois from 8-9 day old embryonated eggs. After 6 days of additiona incubation and for evaluation of each addition passage, bacterial cultural controls were performed on the allantochorion membranes that were harvested. The membrane suspensions were never filtered through bacteriological filters. Earlier investigators had not evaluated the recovery of egg culture material after each egg passage (Liess, and Schimmelpfennig, 1960). Moreover, their recovery method did not differentiate between egg experiments produced with suspicious viral material and unspecific alterations of the chorioallantoic membranes of controls. In the case of non-

sunken membranes, inoculation was accomplished through a triangular window cut out of the egg shell by the addition of virus suspension through the slitted periostracum. Only in controls investigations were the allantochorions sunken.

(C) Tissue Cultures

Detailed descriptions of the preparation of mono-layered tissue cell cultures (Dulbecco and Vogt, 1954; Youngner, 1954; and Bodian, 1956) using embryonal chicken fibroblasts as well as embryonal calf testes tissue can be found in the papers of Berris and Plowright (1958) and Liess, Knocke, and Schimmelpfenning (1960).

(D) Study Animals

For the production of experimental infections, sheep of various ages, which showed no evidence of earlier ecthyma infections, were employed. Animals of white-headed strains were preferred. Skin alterations on the lips of dark-headed sheep cannot be clearly distinguished, particularly during the early stages of infection. Difficulties encountered in procuring study animals did not allow one to make a discriminating selection. As a result, the the possibility of a previously existing immunity in adult animals had to be compensated for.

RESULTS

Cultivation Studies in Embryonated Hen Eggs Using Virus Materals from Humans

Chorioallantoic inoculation with Virus Material H. proved to be a failure after four passages with an average of seven eggs per passage. In the case of individual eggs of the various passages, scattered alterations on the chorio-allantoic membranes were observed. These consisted of small yellowish, round, smooth or nodular, pustule-like protuberances and occasionally smooth opacities or grey-white miliary foci. Increasing numbers of passages had no accelerating

effect on the expression and regularity of these alterations. In a titration experiment performed with Log_{10}- dilutions of a suspensions prepared from a noticeably altered chorioallantoic membrane, a dilution effect could not be discerned. On the contrary, alterations of the type described above were found on the sunken allantochorion membranes in seven out of 17 eggs in a control study.

From a pathological-histological stand point, the opaque areas on the egg allantochorions associated with Virus Material H. were shown to be localized hyperplasia of the ectoderm with expanding epithelial degeneration and weak to moderate inflammation reactions on the mesenchymal tissues. However, the histological examination of nodular alterations on the membranes of several uninoculated eggs from the control study gave similar results[1].

The cutaneous infection of an animal with suspensions prepared from the chorioallantois from the second egg passage did not lead to an ecthyma infection. In spite of this, however, the animal could be infected 45 days later with skin crust material from an experimentally infected sheep.

Cultivation Studies in Embryonated Hen Eggs with Virus Material from Sheep

Macroscopic chorioallantoic alterations of the type seen after inoculation with Virus Material H. from a human, were also seen after inoculation with Virus Material Sheep III. Six passages of 12 eggs per passage were employed in these cultivation studies. No intensification of the membrane alterations could be produced by passage.

Pathologically-histologically in this case also, the alterations on the allantochorion appeared to be composed of epithelial hyperplasia and proliferating cell degeneration in addition to intermediate inflammation of the mesenchymal

1. Dr. H. Schimmelpfennig, Pathological Institute of The Hannover Veterinary School, is thanked for carrying out the histological examinations.

tissues where the alterations consisted of pin head-sized white nodules surrounded by bright areas. Transmission studies with sheep using the egg culture material of this study were not carried out since virus cultivation in cell cultures had already given positive results.

Cultivation Studies with Virus Material from Humans and Sheep in Tissue Culture

The study with embryonal chick fibroblasts did not lead to the desired cultivation of a cytopathogenic virus from study material obtained from both humans and sheep. Likewise, cultivation in cell cultures from calf kidney tissues were unsuccessful. At the time of these studies, a report by Plowright, Witcomb, and Ferris (1959) was published on the cultivation of ecthyma virus in calf testes tissue cultures. Subsequently, monolayer cultures from this cell line were employed and gave good results. The cultivation of ecthyma virus was accomplished without difficulty. Even on the first day post-infection, cytopathic alterations had appeared which led to the complete destruction of the cell layer within a period of four additional days of incubation. In the case of the second virus passage, the same effect was observed after six days. In the subsequent passages, the time for complete cell degeneration ranged from 4 to 6 days. The cytopathic alterations which appeared in calf kidney tissue cultures could be easily differentiated from those in calf testes cultures by direct microscopic examination of the monolayers. The cell alterations occurring in both culture systems consisted of cytoplasmic granulation and slowly progressing rounding-up of strongly light-refractive cells leading eventually to lysis. If the fresh cell cultures were inoculated with virus suspension in a ratio of 1:5 in comparison to the total volume of the culture medium, then there was not uniform degeneration of the culture cells (Fig. 2). In contrast, the production of stagnant infections resulted in a more or less continous number of cells maintaining their spindle-shaped, fibroblastic appearance and appearing to be unaffected by the virus infection. The harvest of virus from such a tissue

culture 6 days post-infection produced a titer of 10^4 to $10^{4.5}$ particles per 0.2 cm^3 of culture medium.

Fig. 1. Cytopathic alterations by ecthyma virus in cell cultures of calf testes tissues . (Native preparation; bright field, 75 X).

Upper right: Alterations at 2nd day post-infection (human virus strain - 15th culture passage)

Upper left: Normal uninoculated cell culture (Control).

Lower right: Total destruction of cell lawn at 3rd day post-infection (human virus strain - 15th cell passage).

Lower left: Total destruction of cell lawn at 3rd day post-infection (Ovine strain - 31st culture passage)

(The reader is referred to the original paper for an actual picture of this figure).

Fig. 2. Cytopathic alterations by ecthyma virus (Human virus Strain) in calf testes tissue. (Native preparation, bright field, 225 X).

Left: Normal uninoculated cell culture (control).

Right: Slowly progressing degeneration with granulation and sphericle formation leading to strong light refraction by the culture cells at 5 days post-infection (see text).

(The reader is referred to the original paper for an actual picture of this figure).

Plate 1

Figs. 3 and 4. Ecthyma contagiosum in humans, 9 -11 days after transmission from sheep.

Figs. 5 and 6. Ecthyma contagiosum in sheep after cutaneous infection on the lips with the tissue culture virus from humans (15th cell passage).

Fig. 5. Vesiculo-pustulosum stage (6 days post-infection).

Fig. 6. Start of typical pustular dermatitis stage (9 days post-infection).

(The reader is referred to the original paper for an actual picture of these figures).

Acceleration of the cytopathic alterations in cells in tissue culture was achieved by reducing the volume ratio of fresh cell culture medium to virus inoculum. If a mixture of 1:1 (1 part virus-containing culture medium of a virus passage to 1 part fresh medium) is added to the cell lawn of a young, uninoculated culture, then the time for complete destruction of the cell lawn can be reduced to 2 to 3 days post-infection. When this was done, 2 to 3 days after inoculation, the formation of cytopathic alterations could be seen in cells which were strongly light-refractive and for the most part adhered to the sides of the culture vessel (Fig. 1). Apparently, the acceleration of the degenerative processes was a manifestation of an increase in virus titer. The employment of young cell cultures for initiation of new viral passages was found to be necessary since in older cultures, the plaque-like degenerative foci did not have significant propagation tendencies. For the purpose of demonstrating that the virus strain EcS VII, which had been passed in calf testes culture over thirty times, was the agent of Ecthyma contagiosum, at certain passage intervals, reverse transmission studies were carried out by inoculating the scarified lips of sheep with undiluted virus-containing tissue culture fluids. The results of the reverse transmission studies will be described later.

The successful cultivation of ecthyma virus from research materials of sheep using calf testes cultures followed similar studies with ecthyma-bearing skin pustule materials of human origin (Virus material M.). The nature of the cellular alterations observed in the latter case could not be differentiated microscopically from those caused by the ovine virus (Fig. 1). After the human virus had been passed 10 times in calf testes cell culture (Virus strain EcM), infection studies in sheep were carried out in order to characterize it. It should be emphasized that a comparative study of virus strains isolated from sheep and from humans could not be carried out using development times and the

type of virus-induced cellular alterations as criteria. The histological comparison of fixed, stained tissue culture preparations, even after employment of histochemical detection methods, required intensive study. In order to identify these virus strains, it was considered necessary in the course of the work to carry out experimental infection studies and cross-immunity tests in sheep. The determination of ether and chloroform resistance served to further characterize the viruses of ovine and human origin which had been grown in calf testes cell cultures. At the same time, determination of these characteristics detected common traits among both strains. Both of the virus strains appeared to be ether-resistance but were sensitive to chloroform when tested using the method of Mayer and Bogel (1961). These testes were carried out qualitatively only without determining decreases in the virus titer.

INFECTION STUDIES IN SHEEP

(see Tables 1 and 2)

(A) Infectious Lip Crust Material from Sheep

Preliminary experiments, in which five study sheep (Nos. II, III, IV, VI, and VII) were infected with ecthyma lipscrust material, verified the infectiousness of materials obtained from naturally diseased sheep as well as the reproducibility of the typical disease symptoms under experimental conditions. In addition to this information, the transmission studies in sheep provided satisfactory infectious material for later laboratory investigations as well as information on the development of immunity and co-determination of symptoms produced by bacterial spores suspected in the skin crust material.

TABLE 1

Experimental Ecthyma Infections in Sheep

study sheep no.	INFECTION origin of virus material	Inoculation procedure and site	Results	Days after first infection	REINFECTION origin of virus material	Inoculation procedure and site	Results
(A) By transmission of infectious skin crusts from sheep							
II	naturally infected sheep	cutaneous lips	++++	81	Sheep No. II	cutaneous lips	no skin lesions
III	Sheep No. II	cutaneous lips	++++	76	Sheep No. II	Cutaneous lips	no skin lesions
IV	Sheep No. II	cutaneous lips	++++	76	Sheep No. II	cutaneous lips	no skin lesions
VI	Sheep No. II	cutaneous lips	++++	Not determined			
VII	Sheep No. II	cutaneous lips	++++	Not determined			
(B) By transmission of cultured virus from sheep							
VIII	18th culture passage	intravenous	no skin lesions	1st 32	15th culture passage	cutaneous lips	++++
				2nd 68	10th culture passage	cutaneous lips	no skin lesions
IX	20th culture passage	cutaneous lips	++++	68	10th culture passage	cutaneous lips	no skin lesions
X	26th culture passage	cutaneous lips	++++	Not determined			
XI	25th culture passage	cutaneous cornea edge	++	Not determined			

Table 1, Continued

Study Sheep no.	INFECTION origin of virus material	Inoculation procedure and site	results	days after first infection	REINFECTION origin of virus material	inoculation procedure and site	results
XII	30th culture passage	cutaneous foot pad	++	Not determined			
XIII	30th culture passage	cutaneous lips	++++	Not determined			
		(C) By transmission of cultured virus from humans					
XIV	9th culture passage	cutaneous lips	+++	not determined			
	9th culture passage	cutaneous lips	++	Not determined			

Explanation of Symbols:

\+ = papulous skin lesions
++ = vesicular-pustular skin lesions
+++= weak scabby skin lesions
++++ = strong scabby skin lesions

TABLE 2

Cross Immunization Studies in sheep with human and ovine ecthyma virus strains grown in calf testes cell cultures

study animal no.	INFECTION designation and origin of virus materials	Inoculation procedure and site	results	days after first infection	REINFECTION designation and origin of virus materials	Inoculation procedure and site	results
XVI	strain EcSVII (ovine) 29th culture passage	cutaneous lips innner leg (right)	no skin lesions	21	strain EcM (human) 12th culture passage	cutaneous lips inner leg (left)	no skin lesions
XVII	Strain EcM (human) 10th culture passage	cutaneous lips	++++	21	Strain EcS (ovine) 31st culture passage	cutaneous lips	no skin lesions
		Inner leg (right)	+++			Inner leg (left)	
XVIII	Strain EcS (ovine) 31st culture passage	cutaneous lips	++++	21	Strain EcM (human) 15th culture passage	cutaneous lips	no skin lesions
		inner leg (left)	+++			inner leg (right)	

+++ = weak scabby skin lesions

++++ = strong scabby skin lesions

(B) Cultured Virus from Sheep

Infection studies were carried out in a total of six sheep using the virus strain Ec S VII which was cultured from virus material obtained from Sheep No. VII. In all the cases, the characteristic ecthyma symptoms were observed. Symptomatically, no differences could be observed in sheep which had been infected with the native skin material. In all the sheep during the blood stage, the dermal infection on the lips led to the formation of scabs with a papillomatotic appearance. In the case of the cutaneous infection on the edge of the cornea (Sheep No. XI) and in the foot pads (Sheep No. XII) the disease stages were less distinct than was the case with infections on the lips. A decrease in the virulence of the virus strain Ec S VII could not be observed even after 30 culture passages. The behavior of Sheep No. VIII is worthy of note. In this case, no disease symptoms were elicited after intravenous injection of 2 cm^3 of virus-bearing tissue culture fluid. The same virus material injected at the same time into Sheep No. IX, however, elicited the development of the typical disease symptoms. In addition, the intravenous application of virus in the case of Sheep No. VIII provided no protection against re-infection which was attempted 32 days later and led to distinct ecthyma lesions.

(C) Cultured Virus from Humans

Two fattened rams were used as the study animals, only one of which showed skin lesions with pustule formation and slight scabbing after cutaneous infection on the lips. A spreading tendency over the site of scarification was not observed as was the case in the earlier transmission studies with virus material obtained from sheep. It was necessary, therefore, to repeat the animal studies with the tissue culture virus from humans. These studies are shown in Table 2. Study sheep No. XVI was refractive to the ovine virus strain at the beginning of the studies and was also refractive to the human virus strain 21 days later.

On the other hand, Sheep No. XVII responded to the first infection with the virus strain Ec M (human) on the lips and on the inner side of the leg. In this case, there was the formation of scabby stages with typical ecthyma characteristics. At the infection site on the inner leg, however, the skin necroses and scab formation were of a lesser magnitude than those on the lips. When a re-infection with the ovine virus strain Ec S VII was attempted 21 days later, this animal was found to be immune. The same material, however, was capable of infecting another sheep (No. XVIII). These animals were no longer capable of being infected with the human virus strain 21 days later.

DISCUSSION OF THE RESULTS

The above described studies on the clarification of the casual connection between the pustular, pock-like skin lesions observed in man which suggest milker's nodules and infectious pustular dermatitis (Ecthyma contagiosum) of sheep and its important sequelae permit the following conclusions:

1. The clinical manifestations in naturally infected sheep as well as those observed in sheep infected with scab material can be compared to those described in the literature leaving no doubt as to a diagnosis of Ecthyma contagiosum ovis. A previous contact of the human patient with sheep in an ecthyma-suspected her confirms the suspicion of the transmission of the infection. Thise suspition is confirmed when one compares the anamnestic facts of the disease in the person concerned with those in the literature.

3. Cross-immunity studies with the ovine virus isolated by cultural techniques from scab material after two experimental sheep passages and with the human virus strain isolated from human pustular material and grown in calf tested culture showed the immunological identity of both virus strains. The virus of human origin causesthesame characteristic ecthyma lesions in sheep as does the ovine virus.

4. The cultural behavior of the human virus strains resembles that of ovine strains in calf testes cell cultures in the following ways:

(a) Time to the appearance of cytophatic effects and to total destruction of the cell lawn under different conditions.

(b) Type of cellular degeneration as observed under the microscope with native preparations.

(c) Sensitivity to chloroform.

(d) Resistance to ether.

5. With regards to pathogenicity for sheep, the human and ovine strains has similar characteristics.

The demonstration, that the ecthyma virus isolated from humans is pathogenic and is the cause of pustular skin lesions in humans, can be carried out by transmission studies to sensitive persons.

Unfortunately, transmissions of this kind were not successful in two study subjects. In both cases, tissue culture fluid from the 10th virus passage of the human ecthyma virus strain was rubbed into the scarified skin on a finger. No skin alterations of any significance were observed later. In the case of both of the study subjects, werare dealing with veterinarins who had been active in veterinary medicine for 3 and 5½ years respectively before the study. Both could recall having exanthema of unknown etiology during this period. Thus, the possibility exists that they had had ecthyma infections at some earlier date and were still immune.

Thus, in the course of this work, the question, posed by Carne, Wickham, White, and Lockley (1946) remains unanswered, that is, whether or not contact with ecthyma-infected sheep activates a latent virus in the persons, or whether the ecthyma virus plays an important etiological role. The above mentioned australian authors, however, believed that the last possibility answered their question.

When the virus of Ecthyma contagiosum is compared to the majority of studies on the pox group of virus, the virus-induced alterations produced in calf testes cell cultures resemble those produced by members of the pox group. For example, the alterations produced by vaccinia virus in calf kidney cultures, which were described by Herrlich and Mayr (1960) are quite similar. However, the ecthyma virus does not resemble the vaccinia virus with regards to the production of pustular alterations on the chorioallantoic membrane of embryonated chicken eggs. On the other hand, it has this characteristic in common with the para-vaccinia virus. Studies on the cultivation of the latter in bovine tissues are not known of. Cultivation in HeLa cell cultures was not successful (Wheeler and Cawley, 1957). It should also be mentioned that the adaption of ecthyma virus to HeLa cells also was unsuccessful, whereas in cultures of monkey kidney tissues, after a single passage one of the vaccinia viruses gave typical cytopathic effects[2].

According to the classification scheme devised by Cooper (1961) for the various groups of virus, the ether resistance of the ecthyma virus places it in the animal pox group along with the vaccinia virus. The chloroform resistance test (C.R. test) recommended by Mayr and Bogel (1961) as an approximate differentiation method for virus types of low and high sedimentation constants has been proven correct considering the chloroform sensitivity of the ecthyma virus.

Additional information on the properties of the ecthyma virus as well as the immune response in experimental animals were obtained during the course of transmission studies with sheep.

In the case of vaccinia virus infections of humans, Herrlich and Mayr (1960) showed the casual connection of neutralizing antibody with immunity. They noticed that there was usually a relatively low level of neutralizing antibody in persons with vaccinia infections. In several cases, no antibody of this type was detected at all, however, it could not be shown that none was present. Since Herrlich and Mayr (1960) noticed that in the case of vaccinia infections, the

immunity was mostly the result of humoral antibody, then the question arises as to what is the nature of the immunity in the case of ecthyma infections.

The relatively limited studies carried out for the detection of neutralizing antibody from experimentally infected animals were conducted using either the virus dilution method (virus dilution factor 10) or using the serum dilution method (serum dilution factor 2 in the case of a virus dosage of ca. 100 KID_{50} = 100 x 50 % culture infectious dose). Approximately 20 blood sera obtained from Study sheep No. VI, VII, VIII, and IX were studied. These were collected before the first infection and after various periods of time up to 30 days (sheep No. VI and VII) or 78 days (sheep Nos. VIII and IX) after the first infection. With none of the methods employed, diagnostically significant titers of neutralizing antibody could not be detected either during the illness or during the convalescent stage. The relatively limited number of study sheep and serum samples does not justify making a final conclusion with regards to the appearance of humoral antibodies or of the lack thereof during the course of ecthyma infections. However, these results agree generally with those obtained by Plowright, Witcomb, and Ferris (1959). These investigators were able to detect a neutralization index of 2.4 in only one of seven experimentally infected sheep after 21 days. In the all the rest of the animals, the titers were between 1.6 and 0.5 which could hardly be considered as significant according to the authors.

During our studies, an attempt was made to infected Sheep No. VIII intravenously with a high dose of ecthyma culture virus (about 100,000 KID_{50}). In spite of this, 33 days later, no neutralizing antibody could be found in the serum. These results throw a significant light on the pecularities of ecthyma infections of sheep and on the immune responses associated with them.

In spite of the question raised, in the course of dermal primary infections of sheep, a relatively stable immunity develops which could be detected in all cases of experimentally induced infected described in this report, and in the literature. However, in the case of intravenous introduction of virus, Study sheep No. VIII was still completely susceptible to re-infection 33 days later. Therefore, it can be concluded that in spite of intensive interaction of the virus with the reticuloendothelial system after intravenous introduction, no humoral antibodies leading to immunity development nor is there any immunity in the skin. From these results it can be seen that the ecthyma virus is dermo- or epidermotropic but not epitheliotropic. Also obvious is the inability of the virus to elicit an immune response in cases not involving localized infections of the skin.

In contrast to infections produced by other virus species of the pox group, ecthyma infections do not show any generalized tendency to spread to other body regions via the blood-lymphatic system and to produce typical skin lesions there not to mention diseases of the internal organs. In the case of sheep, this characteristic at least differentiates ecthyma infections from infections caused by sheep pox.

In contrast, during the course of ecthyma infections in humans, occasionally indications of a limited involvement of the lymphatic system can be demonstrated. In this regards, the reports in the literature as well as several observations of a patient, who reported swelling and tenderness of the axillary lymph nodes, indicate that this is not the result of a concurrent bacterial infection.

Finally, one can show that the virus Ecthyma contagiosum possesses several characteristics which are mutually shared with other members of the pox group. In other ways, however, it occupies a special position within this group. Investigations into the relationship of paravaccinia to Ecthyma contagiosum would appear to be important for the etiological clarification of milker's nodes per se

and milker's nodes-like diseases of the skin in humans. The ability to cultivate ecthyma virus may be found to be profitable in this respect. Additional experimental studies appear to be necessary in order to disclose ultimately any antigenic relationships between the ecthyma virus and the vaccinia virus which is quite important from a practical viewpoint. As a result, the study of the relationship of the ecthyma virus to the pox group and its place within this group would be important not only from an academic viewpoint.

SUMMARY

Ecthyma virus (contagious pustular dermatitis virus) of humans as well as ovine (sheep) origin have been isolated and serially passed in tissue cultures of calf testes cells. After growth in tissue culture, the sheep virus strain was shown to be the contagious agent that causes ecthyma by transmission studies to sheep. Cross-immunity tests were carried out with the sheep virus strain and a human cytopathogenic agent suspected to the ecthyma virus. The human strain was derived from a veterinary student who had recently been in contact with naturally-infected sheep and later developed pustules on his finers which resembled milker's nodes. Three other cases of human ecthyma infections, susposedly contracted from the same animal source, were observed and have been reported on. By means of cross immunity tests in sheep, the immunological identity of both the ovine and human virus strains could be demonstrated. No differences with regards to cultural characteristics and the resistance or sensitivity to ether or chloroform respectively could be demonstrated. Neutralization tests on blood sera taken during the acute and convalescent stages of experimentally-induced disease in sheep did not reveal any significant increase in the specific antibody titer. Based on these

experimental results, immunity in contagious ecthyma of sheep is discussed.

LITERATURE

See Zentralblatt fur Bakteriologie (Journal for Bacteriology) I. Reviews (Referate), 183: 287-301 (1962).